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Metabolic Adaptations of Several Species of Crustaceans and Molluscs to Hypoxia: Tolerance and Microcalorimetric Studies

WILLIAM B. STICKLE, MARTIN A. KAPPER[1], LI-LIAN LIU, ERICH GNAIGER[2], AND SHIAO Y. WANG[3]

Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803

Abstract. Although some species of fish, crustaceans, and molluscs may behaviorally avoid hypoxic masses of small size and limited duration, others cannot. In a series of crustaceans, tolerance of hypoxia over 28 days at 30°C, decreases as follows: *Eurypanopeus depressus* (38 Torr = LC_{50}) > *Palaemonetes pugio* > *Rhithropanopeus harrisii* > *Penaeus aztecus* > *Callinectes sapidus* (121 Torr = LC_{50}). *Callinectes sapidus* and *E. depressus* die during 12-h exposure to anoxia and their heat dissipation rates (quantified by microcalorimetry) are depressed in seawater at 25% air saturation (normoxia) to only 32 and 47% of their metabolic rate at normoxia. In contrast, starved *Crassostrea virginica* and *Thais haemastoma* are anoxia tolerant; their metabolic rates are depressed under anoxia to 75% and 9% of the normoxic rate. Hypoxia tolerance is greater at 20°C than at 30°C for *Penaeus aztecus* and *Crassostrea virginica*, but no temperature effect on tolerance exists for *Callinectes sapidus*. Hypoxia tolerance varies inversely with salinity for *Penaeus aztecus* at 20° and 30°C and for *Callinectes sapidus* at 30°C, but it varies directly with salinity at 20°C in *Callinectes sapidus*. Greater depression of metabolic rate occurs in molluscs during anoxia exposure (and is correlated with greater hypoxia tolerance) than occurs in *Callinectes sapidus* and *Penaeus aztecus*, which are not anoxia tolerant. Heavy mortality probably occurs in young *Callinectes sapidus* and *Penaeus aztecus* and in stages of the life history when the organisms are incapable of avoiding hypoxic water masses.

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[1] Present address: Department of Zoology, Iowa State University, Ames, Iowa 50011.

[2] Permanent address: Cyclobios, Institut fur Zoologie, Abeilung Zoophysiologie, Universitat Innsbrook, A-6020 Innsbrook, Austria.

[3] Present address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831.

Introduction

Mass mortality of marine and estuarine benthic communities due to hypoxia has been widely reported (Santos and Simon, 1980a, b; Harper *et al.*, 1981; Officer *et al.*, 1984; Rabalais *et al.*, 1985). The occurrence of hypoxic bottom waters off the Louisiana coast is a common, recurrent, virtually annual phenomenon, locally known as "dead water" (Bedinger *et al.*, 1981; Turner and Allen, 1982; Boesch, 1983; Renaud, 1985; 1986a; and Rabalais *et al.*, 1986a, b). Hypoxic water masses may persist for weeks. Reports suggest that fish and crustaceans avoid hypoxic waters (Pavela *et al.*, 1983). Juveniles of two species of shrimp, *Penaeus aztecus* and *Penaeus setiferus*, are capable of detecting hypoxic waters and initiating a pattern of avoidance behavior (Renaud, 1986b). Others have suggested that crustacean mortality may be taxon specific, and that hypoxia heavily affects the more susceptible forms (Garlo *et al.*, 1979). The dissolved oxygen concentrations of offshore bottom waters have been observed to be positively correlated with the combined catches of *Penaeus aztecus* and *Penaeus setiferus*, and with fish biomass (Renaud, 1986a).

The tolerance and the physiological and behavioral responses of benthic and demersal invertebrates to long-term (weeks) exposure to hypoxic water is poorly understood. Many studies of tolerance, physiology, and biochemistry are carried out only for hours to a few days. During environmental anoxia, metabolism in bivalves is reduced to a relatively greater extent than in crustaceans, suggesting that bivalves should tolerate long-term anoxia

better than crustaceans (Gade, 1983; Gnaiger 1983a, 1987).

The objectives of this study are: (1) to compare the hypoxia tolerance of five species of decapod crustaceans with that of the oyster, *Crassostrea virginica;* (2) to compare the effects of temperature and salinity on the hypoxia tolerance of *Callinectes sapidus, Crassostrea virginica,* and *Penaeus aztecus* since these species spend all or part of their lives in the estuarine environment; and (3) to correlate the hypoxia tolerance of each species [as well as that of *Thais haemastoma* (see Kapper and Stickle, 1987)], with the degree of depression of metabolic rate during exposure to hypoxia and anoxia.

Materials and Methods

Collection and maintenance

Specimens of all five species of crustaceans used in this study, and *Thais haemastoma* were collected in the vicinity of Grand Isle, LA. Crustaceans studied include the blue crab (*Callinectes sapidus*), the brown shrimp (*Penaeus aztecus*), the xanthid crabs (*Eurypanopeus depressus* and *Rhithropanopeus harrisii*), and the grass shrimp (*Palaemonetes pugio*). *Crassostrea virginica* was purchased at dockside in the same area. Almost all of the specimens were obtained in May or June, a time when the water temperature increased from 21 to 30°C. American oysters (*C. virginica* used in the 10°C experiments) were collected in late October. Specimens were returned to the laboratory in Baton Rouge (LA) where they were adapted stepwise to the experimental water temperatures and salinities. Water temperature was maintained at the desired value (±0.5°C) by placing the experimental aquaria in a constant temperature water table. Experimental salinity (±0.5‰S) was maintained by determining the salinity of Instant Ocean™ artificial seawater (ASW) with a refractometer, and adding either deionized water or an ASW made to 40‰S.

Details of the hypoxia bioassay system are given in Kapper and Stickle (1987). Briefly, each experimental chamber consisted of an aquarium (38 l), containing an undergravel filter overlaid with oyster chips. Seawater was pumped through the aquarium at a rate sufficient to ensure the water was completely exchanged several times per day. Bottled nitrogen, oxygen, and carbon dioxide were mixed with Matheson gas mixers to produce a defined mixture of desired P_{o_2} and pH. This air mixture passed through the undergravel filters at target oxygen tensions of 107, 53, 15, and 0 Torr. Ambient air was used to drive the undergravel filters of the control tanks (142–157 Torr). P_{o_2} was always within 10–15% of the target value at the three higher levels, the 15 Torr tanks were within ±5 Torr, and the P_{o_2} of the 0% air saturation tanks was usually in the range of 3–8 Torr. Each aquarium was covered with Plexiglas and the water level was maintained by a constant-level siphon that drained into a filtration unit that received water from all five experimental chambers in a bioassay series at the same temperature and salinity. Experimental conditions were checked in each chamber daily by measuring P_{o_2}, pH, and ammonium concentration (Solorzano, 1969). The pH varied between 7.6 and 8.1 and ammonium levels were consistently below 25 μM.

The range of sizes and the number of individuals used at each P_{o_2} of a bioassay series varied among species: *Crassostrea virginica,* 30–50 mm long, an average of 20 oysters per P_{o_2}; *Callinectes sapidus,* 28–54 mm carapace width, an average of 8 crabs per P_{o_2}; *Eurypanopeus depressus,* 9–16 mm carapace width, with 25 crabs per P_{o_2}; *Palaemonetes pugio,* 16–27 mm total length with 25 shrimp per P_{o_2}; *Penaeus aztecus,* 21–32 mm total length, with 20 shrimp per P_{o_2}; and *Rhithropanopeus harrisii,* 6–11 mm carapace width, with 15 crabs per P_{o_2}.

Animals were selected for each temperature series so as to minimize size differences within and among the temperature treatment. None of the crustaceans was observed to molt during the bioassay experiments.

Survival at each P_{o_2} was determined daily for 28 days for each bioassay series. LC_{50} values—the P_{o_2} at which 50 percent of the organisms were dead on each day—were calculated by the SAS Probit procedure (SAS Institute, 1982), or by the Spearman-Karber technique (Hamilton *et al.,* 1977) if mortalities in at least two of the five P_{o_2}s were not between 0 and 100%. Percent mortality in the control P_{o_2} tanks varied from 0 to 10% for *Crassostrea virginica,* from 0 to 29% for *Callinectes sapidus;* and from 0 to 50% for *Penaeus aztecus.* Control tank mortality, after 28 days exposure at 30°C and 20‰S, was 20% for *Eurypanopeus depressus,* 52% for *Palaemonetes pugio,* and 40% for *Rhithropanopeus harissii.* Abbott's correction was used to correct control tank mortalities. Significant differences in LC_{50} values among species, temperatures, and days were determined by non-overlap of the 95% fiducial limits.

LT_{50} values are the elapsed days of exposure to anoxia until 50% of the experimental animals died. Thus LT_{50} values are a measure of anoxia tolerance only, whereas LC_{50} values measure the degree of hypoxia tolerance.

Metabolic rate determinations

Rates of heat dissipation were determined by perfusion (open-flow) microcalorimetry using a system described by Gnaiger (1983a, b). Rate functions were calculated as joules·g dry $wt^{-1} \cdot h^{-1}$ and were determined at 25°C and 10‰S for *Callinectes sapidus, Penaeus aztecus,* and *E. depressus.* Rate functions were determined at 25°C and 20‰S for *Crassostrea virginica* and *T.*

haemastoma. The size of experimental animals was limited by the size of the perfusion chamber (3.5 cm^3; inner diameter = 11 mm, inner chamber height = 53 mm). Flow rate through the perfusion chamber was 20 $ml \cdot h^{-1}$. This method of determining metabolic rates has the advantage that the sum of metabolism due to aerobic and anaerobic processes can be measured (Gnaiger, 1983a, b). The metabolic rate of each individual was determined over consecutive periods of perfusion with normoxic water, anoxic water, or 25% normoxic water (=39 Torr). The P_{o_2} of the outflow water was >80% of the inflow under normoxia, and <0.5 Torr under anoxia, as measured with a Cyclobios Twin-Flow respirometer connected to the perfusion calorimeter (Gnaiger, 1983b). Hourly rates were determined from rates integrated for each minute of the hour. Steady state rates were calculated from the average of the last six hours of exposure to each experimental condition. Differences in steady state rates of heat dissipation among P_{o_2} treatments were determined by one-way analyses of variance (ANOVA), and specific differences among treatment means were determined by the Students *t*-test (SAS Inst., Inc., 1982).

Results

Hypoxia tolerance

The long term hypoxia tolerance, at 30°C and 10‰S, of the five species of crustaceans and the oyster can generally be divided into two groups. *Callinectes sapidus* and *Penaeus aztecus* were very sensitive to hypoxia with 28-day LC_{50} values of 121 and 123 Torr (79.1 and 80.4% air saturation), respectively (Table I). The remaining species all had 28-day LC_{50} values lower than 60 Torr, and their hypoxia tolerance decreased in the following order: *Eurypanopeus depressus* > *Palaemonetes pugio* > *Rhithropanopeus harrisii* > *Crassostrea virginica*. Similar results are obtained whether the LC_{50} values are calculated as Torr or percent saturation, but the variation in oxygen solubility with temperature and salinity causes the LC_{50} values calculated in terms of oxygen content (PPM) to deviate significantly from values calculated in Torr or percent saturation.

Species differences also appeared in the rate of mortality of the five species of crustaceans, and the oyster, exposed to defined levels of hypoxic or anoxic seawater. The LC_{50} values for *Callinectes sapidus* and *Penaeus aztecus* increased rapidly as a function of time of exposure; most mortalities occurred within two days exposure (Fig. 1). In contrast, LC_{50} values for *Rhithropanopeus harrisii* and *Eurypanopeus depressus* increased slowly with duration of exposure suggesting that these species are more tolerant and also more variable in their sensitivity to hypoxia (Fig. 2). LC_{50} values for *Palaemonetes pugio* increased rapidly to near the 28-day value of 46 Torr (30% saturation) on the second day of exposure with little mortality occurring thereafter (Fig. 2), whereas mortality occurred on the seventh day for *Crassostrea virginica* (Fig. 1). The rate of mortality varied directly with temperature in *Callinectes sapidus*, *Penaeus aztecus*, and *Crassostrea virginica* (Fig. 1). No mortality occurred in oysters exposed to anoxia (3–8 Torr) at 10°C for 28 days (Table I).

The 28-day LC_{50} of the crustacean species appears to be associated with differences in their natural habitats and activity levels (Fig. 3). That is, the xanthid crabs *Eurypanopeus depressus* and *Rhithropanopeus harrisii*, usually associated with oyster reefs, and the grass shrimp *Palaemonetes pugio* are significantly more tolerant to hypoxia than the potentially nektonic *Callinectes sapidus* and *Penaeus aztecus*. The molluscan species tested were more tolerant of hypoxia than the crustaceans (Fig. 1, 2, 3). The average 28-day LC_{50} for the molluscs, at 30°C and 10‰S, was 37 Torr compared with 78 Torr for the crustaceans. Furthermore, the 7-day LC_{50} at 30°C and 10‰S averaged 59% of the 28-day value for the crustaceans (range 32–89%) compared with 29% for the molluscs (range 0–57%), indicating that crustaceans die more rapidly. Although the oysters were very tolerant of hypoxia at 10 and 20°C, they were sensitive at 30°C. But they had spawned just before the 30°C experiment was conducted, which might have increased their hypoxia sensitivity.

Metabolic rate

The rate of heat dissipation was depressed in the species of crustaceans exposed to hypoxia and in *Crassostrea virginica* and *Thais haemastoma* exposed to anoxia, as shown by analysis of variance (ANOVA); but heat dissipation was considerably more depressed in *Thais haemastoma* than in the other species (Table II). There was no significant reduction in the metabolic rate of *T. haemastoma* exposed to hypoxia (ANOVA: Table II). Two each of *Callinectes sapidus*, *E. depressus*, and *Palaemonetes pugio* exposed to anoxia for 12 h in the perfusion microcalorimetry system died during the experiment. *Callinectes sapidus*, *E. depressus*, *Palaemonetes pugio*, and *T. haemastoma* were therefore treated with hypoxic water at 25% air saturation (39 Torr; Figs. 4, 5). Three *Palaemonetes pugio* died upon exposure to 25% normoxic seawater.

When *Callinectes sapidus* and *E. depressus* were exposed for consecutive 12-h periods to normoxic water, hypoxic water (25% air saturation = 39 Torr) and normoxic water, the heat dissipation rates of both species declined markedly upon exposure to hypoxic water (Fig. 4). However, the posthypoxia metabolic rate of *Callinectes sapidus* in normoxic water only returned to 75% of its pre-exposure normoxic rate, while the posthypoxia

Table I

Twenty-eight-day LC_{50} values for several species of crustaceans and molluscs

Species	T (°C)	LC_{50}[a] S (‰)	Torr	% SAT	PPM	LT_{50}[b]
Crustaceans						
Callinectes sapidus	20	10	74 ± 19	47.7	4.08	<1
		20	124 ± 0	79.9	6.44	<1
		30	123 ± 0	79.3	6.03	<1
	30	10	121 ± 0	79.1	5.63	<1
		20	119 ± 0	77.8	5.23	<1
		30	111 ± 0	72.5	4.61	<1
Eurypanopeus depressus	30	10	38 ± 6	24.8	1.76	1
Palaemonetes pugio	30	10	46 ± 6	30.1	2.14	1
Penaeus aztecus	20	10	105 ± 12	67.7	5.79	<1
		20	92 ± 14	59.3	4.78	<1
		30	93 ± 15	59.9	4.55	<1
	30	10	123 ± 0	80.4	5.72	<1
		20	122 ± 0	79.7	5.36	<1
		30	115 ± 0	75.1	4.77	<1
Rhithropanopeus harrisii	30	10	57 ± 18	37.3	2.65	<1
Molluscs						
Crassostrea virginica	10	10	<0	<0	<0	>28
		20	<0	<0	<0	>28
		30	<0	<0	<0	>28
	20	10	27 ± 8	17.4	1.49	20
		20	16 ± 4	10.3	0.83	18
		30	30 ± 5	19.3	1.47	20
	30	10	59 ± 9	38.6	2.75	8
		20	78 ± 18	51.0	3.43	4
		30	120 ± 8	78.4	4.98	3
*Thais haemastoma**	10	10	15	10	1.01	20
		20	8	5	0.50	27
		30	9	6	0.54	20
	20	10	20	13	1.10	18
		20	12	8	0.62	19
		30	29	19	1.43	20
	30	10	13	9	0.61	>28
		20	22	14	1.17	10
		30	19	12	0.79	15

[a] LC_{50} P_{O_2} causing 50% mortality after 28 days of exposure expressed in: Torr ($\bar{x}$ ± 95% confidence limits, where possible; % air saturation (% SAT); content, mgO_2/l (PPM).

[b] LT_{50}: days of exposure to anoxia causing 50% mortality.

* Data from Kapper and Stickle (1987).

metabolic rate of *E. depressus* in normoxic water returned to 101% of its pre-exposure metabolic rate.

The rate of heat dissipation of the *T. haemastoma* exposed to 25% air saturated water (39 Torr) was depressed significantly less relative to the rate under normoxia, than that of the decapods *Callinectes sapidus* and *E. depressus* (Fig. 5, Table II). Thus, *Thais* is a significantly better metabolic regulator than either species of crustacean. Furthermore, the metabolic rates of the two *T. haemastoma* exposed to various combinations of normoxia, hypoxia (39 Torr), and anoxia adjust rapidly to anoxia and exhibit an oxygen debt upon return to normoxic water (Fig. 5).

Changes in the metabolic rates of starved *Crassostrea virginica* (Fig. 6A), as well as in four of the six *T. haemastoma* (Fig. 6C) provided oysters *ad libitum* in the lab prior to their use (Fig. 6C), were examined. The heat dissipation rates of these animals did not return to the pre-exposure normoxic rate during the 12-h post-exposure period in normoxic seawater. The heat dissipation rate of *C. virginica* increased dramatically upon initial exposure to normoxic seawater, then declined to a steady state

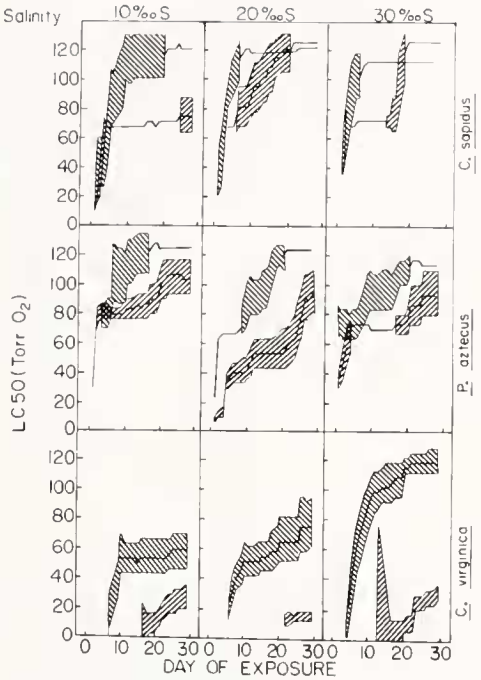

Figure 1. LC_{50} values or the oxygen tension causing 50% mortality (Torr $O_2 \pm 95\%$ fiducial limits), as a function of exposure time of *Callinectes sapidus, Penaeus aztecus,* and *Crassostrea virginica.* Values were obtained over 28 days, at 10, 20, and 30‰S, and at 20 (□) and 30°C (■).

level that was unchanged during 12 h of exposure to anoxic water. Upon the return to normoxic seawater, the heat dissipation rates of the oysters increased dramatically for the first three hours, and then declined to the initial normoxic steady state level. In contrast, the heat dissipation rate of the two apparently fed *T. haemastoma* exhibited an oxygen debt upon reexposure to normoxic water after 12 h of anoxia and then returned to the initial normoxic metabolic rate (Fig. 6B).

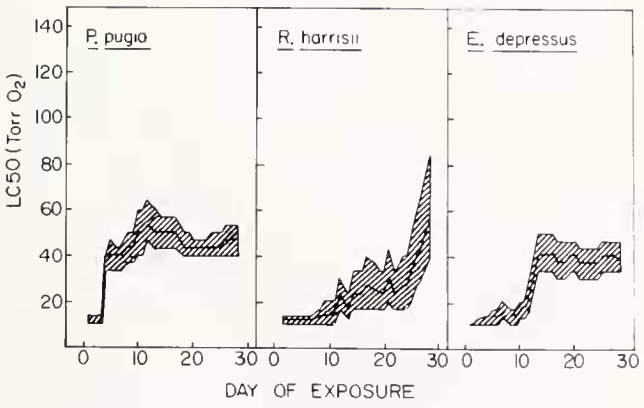

Figure 2. LC_{50} values (Torr $O_2 \pm 95\%$ fiducial limits) as a function of exposure time of *Palaemonetes pugio, Rhithropanopeus harrisii,* and *Eurypanopeus depressus;* 28 days at 30°C and 10‰S.

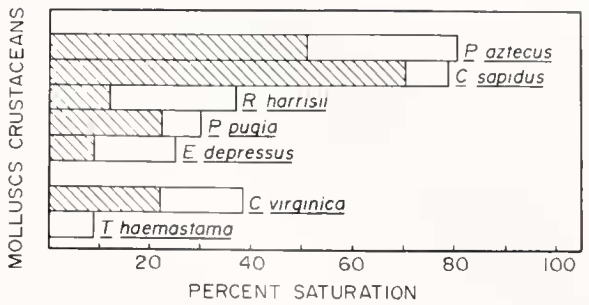

Figure 3. LC_{50} values, expressed in percent saturation, for seven species after 7 days (cross hatched portion of bars) and 28 days (total bar lengths) of exposure at 30°C and 10‰S.

As expected, the magnitude of metabolic rate depression of these crustaceans and molluscs is directly related to their LT_{50}—their mortality upon exposure to anoxia. *Callinectes sapidus, E. depressus,* and *Palaemonetes pugio* have LT_{50} values of one day or less at 20 and 30°C (Table I). Two individuals from each of these species were examined for evidence of metabolic rate depression: none was found, and all the specimens died during the 12-h exposure to anoxia. The metabolic rate of *Crassostrea virginica* exposed to anoxic water was 75% of the normoxic rate, and their LT_{50} was 18 and 4 days at 20°C and 30°C, respectively. The metabolic rate of *Thais haemastoma* under anoxia was reduced to 9% of their rates in normoxic water (Table II) and their LT_{50} was 19 and 10 days at 20 and 30°C and 20‰S, respectively (Table I).

No relationship exists between the degree of metabolic rate depression upon exposure to anoxia, and the 28-day LC_{50} values, which are indicative of hypoxia tolerance. Metabolic rate depression as a function of hypoxia appears to be inversely correlated with hypoxia tolerance. Metabolic rates during exposure to 25% air saturation are reduced to 74% of the normoxic rate for *T. haemastoma,* 32% for *Callinectes sapidus,* and 47% for *E. depressus* (Table II).

Evidence of a classical oxygen debt exists in two *T. haemastoma* upon return to normoxic seawater after exposure to anoxic water (Fig. 5, 6B). This oxygen debt is of short duration in the two oyster drills shown in Figure 5. However, four *T. haemastoma* did not exhibit an oxygen debt upon exposure to normoxic seawater after 12 h to anoxic water (Fig. 6C).

Discussion

The five species of crustaceans and two species of molluscs studied differ in their tolerance to chronic hypoxia, as well as in their sensitivity to acute exposure to anoxia. Among the crustaceans, hypoxia tolerance in each species appears to be closely correlated with activity level

Table II

Steady state rate of heat dissipation (joules · g dry wt^{-1} · h^{-1}) of four species of molluscs and crustaceans under normoxic (100% air saturation), hypoxic (25% air saturation), and anoxic (<5% air saturation) conditions

Species	n	S‰	Normoxia	Hypoxia	%	Anoxia	%
Crustaceans							
Callinectes sapidus	5	10	18.47 ± 0.31	5.91 ± 0.25*	32	N.D.	
Eurypanopeus depressus	5	10	8.70 ± 0.27	4.06 ± 0.46*	47	N.D.	
Molluscs							
Crassostrea virginica	3	20	3.16 ± 0.51	N.D.		2.38 ± 0.47*	75
Thais haemastoma	6	20	8.76 ± 0.99	N.D.		0.78 ± 0.04*	9
	2	20	19.60 ± 10.08	14.90 ± 8.55	76	N.D.	

All metabolic rates were determined at 25°C. N.D. = no data. All crabs died upon exposure to anoxic water. % = Percent of normoxic rate. * = significantly different ($P < 0.05$) from the normoxic rate.

and metabolic rate (Fig. 3). *Eurypanopeus depressus* and *Rhithropanopeus harrisii* are associated with oyster reefs; *Palaemonetes pugio* is associated with salt marsh vegetation; and juvenile *Callinectes sapidus* and *Penaeus aztecus* are active swimmers, migrating between estuaries and offshore waters during their life cycles. A two-fold difference also exists in the metabolic rates of *Eurypanopeus depressus* and *Callinectes sapidus* exposed to normoxic seawater (Table II).

Sensitivity to hypoxia has also been measured as a function of mortality in anoxic seawater, represented by LT_{50} values. The resistance of marine invertebrates to oxygen deficiency is correlated with the natural habitats of the species (Fig. 3; Theede *et al.*, 1969; Theede 1973). In this study, mortality occurred more rapidly in the crustaceans exposed to hypoxia than in the molluscs, when all of the temperature-salinity combinations tested were considered. All of the crustacean species exposed to anoxia had LT_{50} values of one day or less, whereas the LT_{50} values of *Crassostrea virginica* ranged from greater than 28 days at 10°C, to three days at 30°C and 30‰S. Moreover, the LT_{50} of *Thais haemastoma* ranged from greater than 28 days at 30°C and 10‰S, to 10 days at 30°C and 20‰S.

The LT_{50} value is not a very sensitive indicator of hypoxia tolerance, because only animals exposed to anoxia can be included in the calculation of the parameter. In contrast, the determination of a LC_{50} value requires data about survival as well as mortality, from several P_{O_2} treatments, so several degrees of hypoxia are represented. There is no correlation between the LT_{50} and the 28-day LC_{50} P_{O_2}.

If, for the species studied, the LC_{50} values for short term exposure are expressed as a function of the 28-day LC_{50}, the phylogenetic differences between crustaceans and molluscs are clearly highlighted. Thus, day 2 and day 7 LC_{50} values represent 0 and 21% of the 28-day values for the two species of molluscs at 30°C and 10‰S, whereas they represent 32 and 60% of the 28-day value for the crustaceans.

Other environmental factors have an antagonistic effect upon the tolerance of estuarine invertebrates to hypoxia and anoxia. Sensitivity to hypoxia increases with temperature in *Crassostrea virginica* and *Penaeus aztecus,* as well as in *T. haemastoma* (Kapper and Stickle, 1987), and probably results from an increased metabolic rate at elevated temperature. The survival time of oysters, experimentally buried to simulate natural sedimentation events, varied inversely with temperature, from more than 5 weeks at <5°C, to 4 days at temperatures >25°C (Dunnington, 1968). Prolonged exposure of oysters to fresh water and low salinities (<5‰S) has caused heavy mortality because they remained closed and could not feed and maintain an aerobic metabolic rate (Andrews, 1982). Oysters died, presumably because of anoxic conditions produced by dredging which resulted in an oxygen demand of spoil bank sediments and modification of the local hydrographic regime (Hoese and Ancelot, 1987).

Hypoxia tolerance varied inversely with salinity for *Penaeus aztecus* at 20 and 30°C and for *Callinectes sapidus* at 30°C, but varied directly with salinity at 20°C for *Callinectes* (Table I). The inverse relationship between tolerance and salinity noted for *Callinectes sapidus* and *Penaeus aztecus* is the opposite of that expected on the basis of theoretical osmoregulatory costs; but activity patterns associated with feeding may override the energetic costs of osmoregulation. Juvenile blue crabs and shrimp sometimes use the most brackish regions of estuaries as a nursery ground so the inverse relationship between salinity and hypoxia tolerance is correlated with the distribution of life history stages of these species.

Behavioral avoidance activities by juvenile (65 to 101 mm total length) penaeid shrimp may temporarily allow them to escape oxygen deficient water; *i.e.,* below 2.0 ppm (29% air saturation or 45 Torr at 22°C and 22‰S)

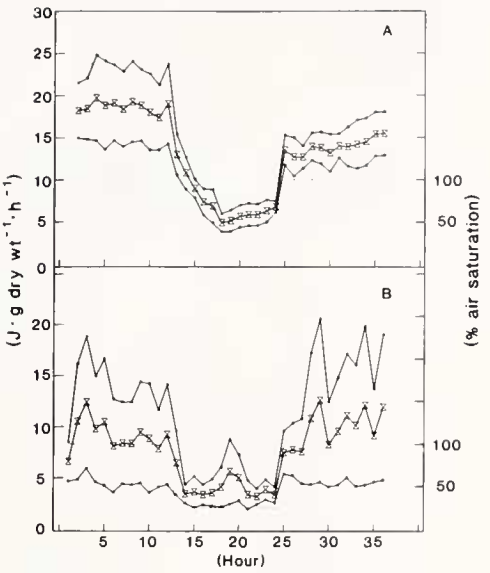

Figure 4. Hourly heat dissipation rates of 5 *Callinectes sapidus* (A) and 5 *Eurypanopeus depressus* (B) at 25°C and 10‰S. Rates expressed as J·g dry $wt^{-1} \cdot h^{-1}$ ± S.E. (mean ⊠ and standard error limits — ● —) for crabs exposed to consecutive 12 h periods of normoxia, hypoxia (25% air saturation), and normoxia. Dashed lines: percent air saturation values for ambient seawater.

for *Penaeus aztecus* and 1.5 ppm (22% air saturation or 34 Torr) for *Penaeus setiferus* (Renaud, 1986b). Juvenile shrimp may be able to alter their migration patterns to move around patches of hypoxic water (Rabalais *et al.*, 1986a, b; Renaud, 1986a), provided the patches are spatially and temporally isolated.

The values for metabolic rate depression observed in this study fall within the range of values reported in the literature and emphasize the need to consider this component of physiological adaptation in relation to the life cycle niche occupied by each species. It is not surprising that juvenile blue crabs, which are active swimmers, are intolerant of anoxia, and that their metabolic rate at 25% air saturation is depressed only to 32% of their normoxic metabolic rate. Although *Eurypanopeus depressus* is also intolerant of anoxia and exhibits metabolic rate depression to 47% of its normoxic rate upon exposure to 25% air saturated seawater, its brackish water oyster reef habitat is not exposed to hypoxic water masses of the same duration as those that develop offshore (Rabalais *et al.*, 1985). These crabs are exposed to diurnal variations in oxygen tension. In contrast, the copepod *Cyclops abyssorum*, which lives in alpine ponds that become hypoxic in the winter, exhibits metabolic rate depression to 17% of the normoxic rate upon exposure to anoxia at 6°C (Gnaiger, 1981).

The metabolic rate depression of molluscs exposed to anoxic seawater is also highly variable among species, ranging from 9% of the normoxic rate in *T. haemastoma* (Table II), 11% in *Mytilus edulis* (Famme *et al.*, 1981; Shick *et al.*, 1983), 75% in *Crassostrea virginica* to only 97% in *Mulinia lateralis* (Shumway *et al.*, 1983). Sessile and infaunal bivalves generally show a strong resistance to anoxia due in part to a reduction in activity and hence energy use (Shick *et al.*, 1986). In this study, *Crassostrea virginica* (2 to 19 mg dry weight) were starved for 35 to 65 days prior to the experiment. In bivalves, their oxygen consumption rate is directly coupled with filtration activity associated with feeding (see discussion by Bayne *et al.*, 1976). Starved *Crassostrea virginica* probably exhibited a reduced filtration activity and heat dissipation rate. In *Mytilus edulis*, the increase in oxygen consumption of starved mussels offered food is almost instantaneous (Widdows, 1973). The increased heat dissipation rate of oysters immediately after perfusion with normoxic seawater (Fig. 6A) may therefore represent "testing" of an altered ambient environment after which the active rate of the oysters was reduced to the standard, nonfeeding rate. *T. haemastoma* is exposed to diurnal and seasonal periods of anoxia, both in the water column, and when it burrows into the anoxic zone of sediments for a large portion of the winter and intermittantly in the summer (Kapper and Stickle, 1987).

The two metabolic patterns shown by *T. haemastoma*

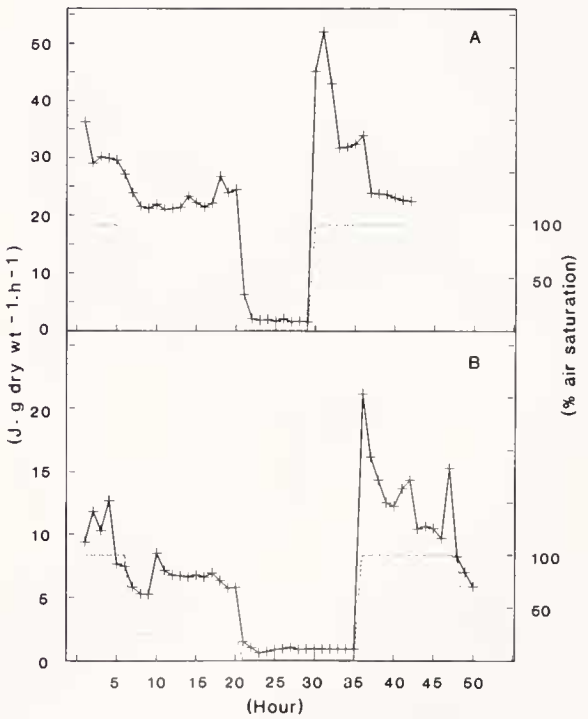

Figure 5. Hourly heat dissipation rates (at 25°C and 30‰S) of two (A and B) *Thais haemastoma* (solid lines —┼—) exposed to various combinations of normoxia (100% air saturation), hypoxia (25% air saturation), and anoxia. Dashed lines: percent air saturation values for ambient seawater.

in response to normoxic seawater after 12-h exposure to anoxic seawater (Fig. 6B, C), are probably related to the feeding history of the snails. Feeding rate is the primary bioenergetic component to become variable in gradients of environmental factors, and certain individuals cease feeding altogether under stressful conditions (Stickle, 1985). Small oyster drills, such as those used in this study, are particularly sensitive to the selection of optimum-sized bivalve prey, because prey size can limit the ingestion rate, and hence the energy budget of the predator (Garton, 1986). *T. haemastoma* prefers a number of different prey items (Butler, 1953), the importance of which may vary with the size of the snail. Oyster drills also exhibit a large specific dynamic action effect, elevated metabolic rates associated with digestion of food, in normoxic seawater, anoxic seawater, and when exposed to the air (Stickle *et al.*, 1986). All of these factors probably contributed to the variability in individual metabolic rates which resulted in two apparent patterns of response in the recovery of oyster drills from 12 h of anoxia.

The metabolic rate depression of *T. haemastoma* exposed to anoxia (Table II) suggests a switch to the relatively more efficient succinate and propionate pathways in the molluscs, compared with the well developed, but less efficient, classical glycolysis system in the crustaceans (Gade, 1983; Gnaiger, 1983a, 1987; and deZwaan and Thillart, 1985). During initial exposure to environmental anaerobiosis, the biochemically estimated ATP turnover rate may drop to about 10% of aerobic resting rates in crustaceans, and the reductions may be even larger in molluscs (deZwaan and Thillart, 1985). During the initial exposure to anoxia, when aspartate is still the precursor of succinate in the molluscs, the rate is three to five times higher than the subsequent anaerobic steady state and is fueled by both phosphagen and ATP hydrolysis (deZwaan and Thillart, 1985; Kapper and Stickle, 1987). When the steady state is reached, the glycolytic flux is reduced and channeled towards malate, whereas the phosphagen pool is somewhat depleted relative to normoxia levels (deZwaan and Thillart, 1985; Kapper and Stickle, 1987).

No evidence of oxygen debt was observed with *Callinectes sapidus* (Fig. 4A), *E. depressus* (Fig. 4B), starved *Crassostrea virginica* (Fig. 6A), or specimens of *T. haemastoma* whose metabolic rates did not even recover to the initial normoxic rate after 12-h exposure to anoxic seawater (Fig. 6C). In contrast, the *T. haemastoma* individuals that did recover to the initial normoxic rate after 12-h exposure to anoxic seawater, exhibited an oxygen debt upon their postanoxic exposure to normoxic seawater (Fig. 6B).

Two basic processes occur during recovery from environmental anoxia: (1) recharging of the phosphagen pool; and (2) the disposal of end products by excretion, oxidation, or conversion back to anaerobic substrates (Ellington, 1983). Oxygen debts are regular phenomena in free-living invertebrates and may be attributed, at the molecular level to the increased energy demands for disposal of end products and recharging of the phosphagen and ATP pools (Herreid, 1980). Patterns of oxygen debt in invertebrates tend to be highly variable from species to species, and may reflect differences in the degree of reduction of energy metabolism, hence end-product accumulation under anoxic conditions (Herreid, 1980) and the duration of exposure to anoxia. Upon exposure to anoxia for 24 h, *Thais haemastoma*, the species that exhibited the greatest metabolic rate depression in our study (Table II), showed a return of the adenylate energy charge to the pre-exposure level within 6 h. But the arginine phosphate concentration returned to only about half of its pre-exposure value 24 h after the oyster drills were returned to normoxic water (Kapper and Stickle, 1987). Lack of an oxygen debt in some *T. haemastoma* (and possibly *Crassostrea virginica*), whose postanoxia metabolic rate did not recover to the initial rate after 12-h exposure to anoxia, may be due to a reduced metabolic

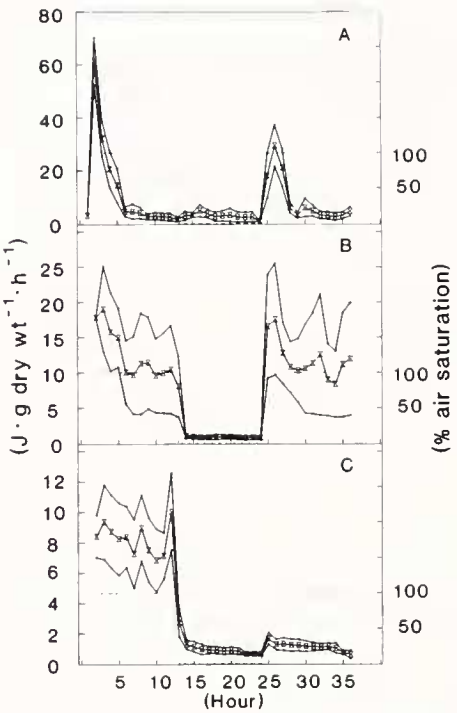

Figure 6. Hourly heat dissipation rates of three *Crassostrea virginica* (A), two *Thais haemastoma* which recovered from exposure to anoxia at 25°C and 20‰S (B), and four *T. haemastoma* which did not recover from exposure to anoxia (C). Rates expressed as: J·g dry wt^{-1}·h^{-1} ± S.E. (solid lines for the mean ⊠; standard error limits by solid lines). The animals were exposed to consecutive 12-h periods of normoxia, anoxia, and normoxia at 25°C and 20‰S. Dashed lines: percent air saturation values for ambient seawater.

rate after long term starvation (Fig. 6C compared with 6B) coupled with a slow replenishment of the phosphagen pool and, perhaps, washout of anaerobic end products during exposure to anoxic water.

In conclusion, significant interspecies variability exists in the 28-day LC_{50} values for the five species of crustaceans studied, ranging from 38 Torr in *Eurypanopeus depressus*, to 121 Torr in *Callinectes sapidus*. In addition, species differences exist in the rate of mortality of the five species of crustaceans, and in the oyster, when exposed to defined levels of hypoxic or anoxic seawater. LC_{50} values for *Callinectes sapidus, Penaeus aztecus,* and *Palaemonetes pugio* increase rapidly during the first two days of exposure, in contrast to those of *Rhithropanopeus harrisii* and *Eurypanopeus depressus* which increased slowly over the exposure period. Sensitivity to hypoxia increased with temperature in *Callinectes sapidus, Crassostrea virginica,* and *Penaeus aztecus,* with salinity effects being less significant. Natural habitat, activity level, and seasonal differences appear to exist in the mortality rate of these five species of crustaceans. Both *Callinectes sapidus* and *Eurypanopeus depressus* died during 12 hours exposure to anoxia with little decline in the metabolic rate, and their metabolic rate in 25% air saturated seawater is reduced to only 32–40% of their metabolic rate under normoxia. In contrast, both *Crassostrea virginica* and *Thais haemastoma* are tolerant of 25% air saturated seawater, with the rate of *Thais haemastoma* being 76% of that under normoxic conditions. Metabolic rate depression occurs in both species under anoxic seawater, to 75% of the normoxic rate in *Crassostrea virginica,* and 9% in *Thais haemastoma.*

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